

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Yoshihiro OHMIYA, et al.

Serial No.: 10/526,569 Art Unit: 1652

Filed: March 4, 2005 Examiner: Hope Robinson

For: Secretory or Membrane-binding chimeric protein

DECLARATION

Honorable Commissioner of Patents and Trademarks

Washington, D. C. 20231

SIR:

I, Yoshihiro OHMIYA, declare that:

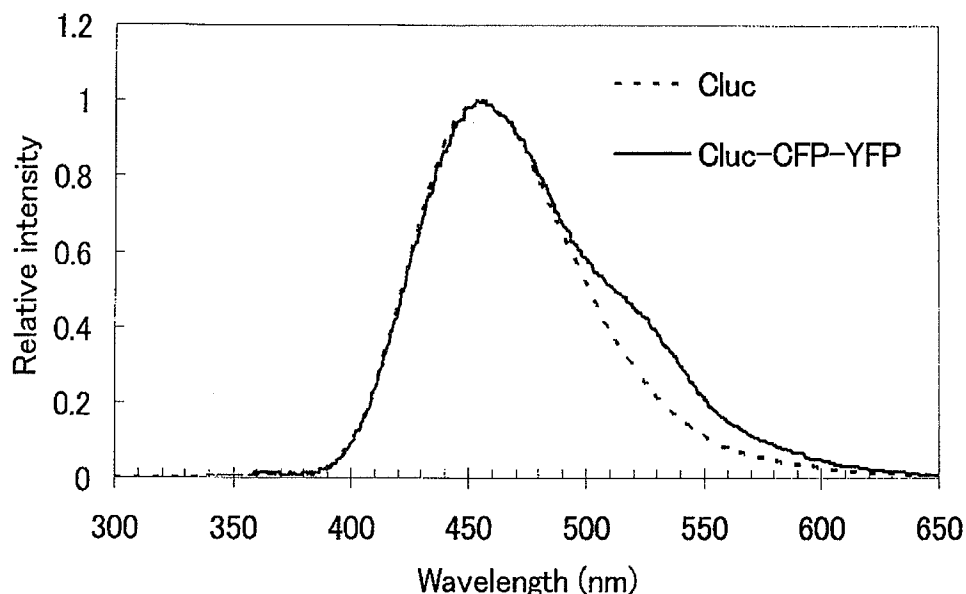
- 1) I am one of the inventors of the above-identified application, and am familiar with the subject matter of said application as well as the disclosures in the cited reference.
- 2) I would like to submit an additional experiment which indicates that an energy transfer occurs between *Cypridina* luciferase and YFP as it occurs between *Vargula* luciferase and YFP.

ADDITIONAL EXPERIMENT

A mutant cyan fluorescent protein (ECFP) gene (Clonotech, PT3258-5) and a mutant yellow fluorescent protein (EYFP) gene (Clonotech, PT3175) were linked to the downstream of a secretory type luciferase

gene derived from *Vargula hilgendorffii* related species *Cypridina noctiluca* (hereinafter abbreviated as "Cluc"; ATTO Corporation, NCBI No.AB177531) to gain a gene construct (SEQ ID NO.1). The gene construct was then inserted into the downstream of CMV promoter to gain pCluc-CFP-YFP vector. The amino acid sequence of the luminescent/fluorescent fusion protein, pCluc-CFP-YFP (116.6 kDa), produced by the said gene is shown in SEQ ID No.2 below. The pCluc-CFP-YFP vector (500 ng) was transfected to NIH3T3 cell using Lipofectamine Plus Reagent. The cell was cultured in DMEM + 10% FBS for 24 hours and then the culture medium was collected. *Vargula* luciferin (1 mM *Vargula* luciferin/ 60 mM phosphate (pH6.4), 20 mM sodium sulfite, 0.3 M sodium ascorbate) was added thereto and the luminescence spectra were measured by LumiFru Spectro Capture AB-1850 (ATTO Corporation).

The result is shown in Fig. 1 below. The light-emitting enzyme alone (Cluc) showed blue light emitting having a peak at 460 nm. On the other hand, the fusion protein had a peak at 530 nm due to the energy transfer from Cluc to YFP. Therefore, it is proved that a luciferase derived from *Vargula* related species *Cypridina noctiluca* can be used as an energy-generating protein of the present invention.



(SEQ ID: No.1)

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ATGAAGACCTTAATTCTTGCCGTTGCATTAGTCTACTGCGCCACTGTTTCATTGCCAGGACTGTC
CTTACGAACCTGATCCACCAAACACAGTTCCAACCTCCTGTGAAGCTAAAGAAGGAGAATGTAT
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TTAGAACATTCTATGGAAAGAGATTCCAGTTCCAGGAACCTGGTACATACGTGTTGGGTCAAGG
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(SEQ ID: No.2)

M	K	T	L	I	L	A	V	A	L	V	Y	C	A	T	V	H	C	Q	D
G	P	Y	E	P	D	P	P	N	T	V	P	T	S	C	E	A	K	E	G
E	C	I	D	S	S	C	G	T	C	T	R	D	I	L	S	D	G	L	C
E	N	K	P	G	K	T	C	C	R	M	C	Q	Y	V	I	E	C	R	V
E	A	A	G	W	F	R	T	F	Y	G	K	R	F	Q	F	Q	E	P	G
T	Y	V	L	G	Q	G	T	K	G	G	D	W	K	V	S	I	T	L	E
N	L	D	G	T	K	G	A	V	L	T	K	T	R	L	E	V	A	G	D
I	I	D	I	A	Q	A	T	E	N	P	I	T	V	N	G	G	A	D	P
I	I	A	N	P	Y	T	I	G	E	V	T	I	A	V	V	E	M	P	G
F	N	I	T	V	I	E	F	F	K	L	I	V	I	D	I	L	G	G	R
S	V	R	I	A	P	D	T	A	N	K	G	M	I	S	G	L	C	G	D
L	K	M	M	E	D	T	D	F	T	S	D	P	E	Q	L	A	I	Q	P
K	I	N	Q	E	F	D	G	C	P	L	Y	G	N	P	D	D	V	A	Y

C K G L L E P Y K D S C R N P I N F Y Y
Y T I S C A F A R C M G G D E R A S H V
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N V D S Y T E V E K V R I R K Q S T V V
E L I V D G K Q I L V G G E A V S V P Y
S S Q N T S I Y W Q D G D I L T T A I L
P E A L V V K F N F K Q L L V V H I R D
P F D G K T C G I C G N Y N Q D F S D D
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D G D V N G H K F S V S G E G E G D A T
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T L V T T L T W G V Q C F S R Y P D H M
K Q H D F F K S A M P E G Y V Q E R T I
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L V N R D I E L K G I D F K E D G N I L G
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E L D G D V N G H K F S V S G E G E G D
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D N H Y L S Y Q S A L S K D P N E K R D
H M V L L E F V T A A G I T L G M D E L
Y K L E

I, the undersigned, declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: Nov. 27th. 2007.

Y. C.

Yoshihiro OHMIYA